ASD TECHNICAL REPORT 61-674

TOXICOLOGY OF AURIN TRICARBOXYLIC ACID AND ITS ANTIDOTAL EFFECTIVENESS AGAINST BERYLLIUM

MAURICE E. KING

ARMOUR RESEARCH FOUNDATION

DECEMBER 1961

CONTRACT No. AF 33(616)-6947

BIOMEDICAL LABORATORY
AEROSPACE MEDICAL LABORATORY
AERONAUTICAL SYSTEMS DIVISION
AIR FORCE SYSTEMS COMMAND
UNITED STATES AIR FORCE
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

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CONTRACT No. AF 33(616)-6947
CONTRACT MONITOR: KENNETH C. BACK, PH.D.
PROJECT No. 7165
TASK No. 716501

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900 - February 1962 - 20-857 & 858

FOREWORD

This program is entitled "Toxicology of Aurin Tricarboxylic Acid and Its Antidotal Effectiveness Against Beryllium." It was conducted at Armour Research Foundation of Illinois Institute of Technology, Technology Center, Chicago 16, Illinois, under the sponsorship of the Aerospace Medical Laboratory of Aeronautical Systems Division. The program was initiated and monitored by Capt. D. Miller, USAF, Dr. K. C. Back, and Dr. A. A. Tamas of the Toxic Hazards Section, Physiology Branch, Biomedical Laboratory, Aerospace Medical Laboratory. Work was conducted under Contract No. AF 33(616)-6947, Project No. 7165, "Health Hazards of Material and Radiation," Task No. 716501, "Evaluation and Control of Toxic Chemical Materials." At Armour Research Foundation the research program was designated ARF Project C 170. Dr. M. E. King, Dr. R. Ehrlich, Mr. A. Shefner, Mr. J. Kyle, and Mr. V. Greene planned and conducted the program. Mr. L. Luther, Mr. E. Allen, and Mrs. V. Martin also supported this research effort. Pathological examinations were made by Dr. W. F. Eisenstaedt.

This has been designated Report No. ARF 3170-6. The studies performed and compiled into this report were conducted during the period of March 1, 1960, to August 31, 1961.

Animal experimentation was conducted in accordance with the Rules for Animal Care as established by the American Medical Association.

The publication of this report does not constitute approval by the Air Force of the findings or conclusions contained herein.

ABSTRACT

Monkeys and dogs were used in a series of studies designed to assess the ability of aurin tricarboxylic acid (ATA) to provide protection against acute beryllium poisoning. The acute LD_{50} of ATA was found to be 344 mg/kg for monkeys and 164 mg/kg for dogs. Neither species exhibited significant hematological changes when given weekly ATA doses of 25 mg/kg over an 8-month period.

The lethal intravenous dose of beryllium sulfate was 0.6 mg/kg for both dogs and monkeys, but the value increased to between 1 and 3 mg/kg when given by intratracheal injection. Acute toxic effects were not observed by either intravenous or intratracheal doses of suspensions of beryllium oxide.

Treatment with ATA appeared to have therapeutic value in monkeys exposed to beryllium, but no significant response was observed in dogs.

PUBLICATION REVIEW

Jos M Quashnock

JOS. M. QUASHNOCK
Colonel, USAF, MC
Chief, Biomedical Laboratory
Aerospace Medical Laboratory

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I. INTRODUCTION

Studies were conducted to assess the effectiveness of aurin tricarboxylic acid (ATA) as an antidote for beryllium (Be) poisoning in dogs and monkeys and to determine the feasibility of using ATA in cases of accidental human exposure to Be. During the program the following toxicity studies were conducted: acute and chronic toxicity of ATA by intravenous administration, acute toxicity of beryllium sulfate ($BeSO_4$) by intravenous and intratracheal administration, acute toxicity of beryllium oxide (BeO), and toxicity of the ATA-Be complex. The therapeutic effectiveness of various ATA treatment schedules was investigated after the initial toxicity studies.

II. EXPERIMENTAL WORK

A. Animals and Materials

The male and female rhesus monkeys used in these studies were obtained from Shamrock Farms, Incorporated, and weighed from 1.5 to 2.4 kg. Two monkeys were kept in each cage and were fed Purina monkey chow. The dogs used were male mongrels obtained from the city pound and weighed 8 to 15 kg. They were fed Purina dog chow. Both species were quarantined for 2 weeks and given kidney and liver function tests as well as hematological examinations before they were used in the experiments.

Solutions of ATA were prepared by dissolving crystals of the repurified ammonium salt (Eastman Chemical Products, Incorporated) in distilled water and adjusting to pH 7.3 with sodium hydroxide. Beryllium sulfate solutions were prepared by dissolving the tetrahydrate crystals (Fisher Scientific Company) in distilled water with no adjustment of pH. Because of its insolubility in water, suspensions of low-fired BeO (E. H. Sargent Company) were prepared in 0.15% carboxymethylcellulose by stirring in a Vertis high-speed homogenizer.

B. Acute ATA Toxicity

The LD_{50} for intravenous administration of ATA was determined for both dogs and monkeys. The animals were weighed and then injected with the required amount of 10% or 20% ATA and returned to their cages for observation. Animals which survived for 7 days were considered survivors in the calculation of LD_{50}.

Immediately after the injection, all the animals appeared weak and a general decrease in activity was observed. Vomiting and diarrhea occurred within 1 hr in all the dogs and in the majority of the monkeys. The clotting time of the blood was noticeably increased as evidenced by continued bleeding at the site of

the injection. Blood was found in the urine of one monkey. However, this animal survived the 7-day period and no significant pathological changes were observed when the animal was sacrificed. Four dogs developed open wounds at the injection site. Although they survived, they were unusually docile during the observation period. In general, animals which succumbed to ATA were inactive until death, while animals which recovered were inactive for about 48 hr after injection.

Because of the variation in survival time, especially in monkeys, the time of death could not be correlated with the dose received. For example, of the five monkeys given 400 mg/kg, one survived the 7-day period, while the others died after 5 days, 3 days, 1 day, and 5 hr. Except in three cases, death in dogs occurred within 24 hr.

After the 7-day observation period the survivors were sacrificed and autopsied. Animals which succumbed to ATA were autopsied shortly after death. Both gross and microscopic pathological changes were noted in the animals. Table 1 summarizes the dosage and mortality of the monkeys and the dogs.

The pathological data give no clear-cut cause for death. Some tissue damage occurred in the animals that died as a result of the ATA and in those that were sacrificed after the observation period. Generally the liver and kidneys were most severely damaged, more so in dogs than in monkeys. However, it cannot be unequivocally stated that damage to these organs was the primary cause of death.

The LD_{50} values and the standard error were determined by probit analysis.[1] These values are 344 $\pm$ 20.3 mg/kg for monkeys and 164 $\pm$ 13.5 mg/kg for dogs. The equations for the regression curves are:

$Y = -23.52 + 11.24\,X$ for monkeys

$Y = -10.19 + 6.56\,X$ for dogs.

The value for monkeys agrees with that obtained by Schubert and Rosenthal[2] for rats -- 340 $\pm$ 8 mg/kg. No reason can be given for the apparent greater toxicity of ATA for dogs. This increased toxicity may perhaps be correlated with the more extensive pathological damage in dogs.

C. Chronic ATA Toxicity

Two dogs and two monkeys were given for 8 months weekly intravenous ATA injections of 10 and 25 mg, respectively. Weekly hematological examinations showed no significant change in red and white counts, differential count, hematocrit value, and clotting time. Although still within the normal range, higher values for white counts were found. The differential counts, however, did not show a consistent increase of any one type.

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[1] Finney, D. J., Probit Analysis, Cambridge Univ. Press, 1952.

[2] Schubert, J. and M. W. Rosenthal, Arch. Ind. Health, Vol 19, p 169, 1959.

Table 1

SURVIVAL OF DOGS AND MONKEYS INJECTED WITH ATA

Monkeys		Dogs	
ATA, mg/kg	Survival Time	ATA, mg/kg	Survival Time
81	7 days	77	7 days
200	7 days	87	6 days
200	7 days	95	7 days
278	6 hours	120	7 days
308	7 days	120	7 days
325	2 days	120	7 days
325	7 days	120	7 days
325	7 days	120	7 days
325	7 days	140	1 day
325	1 day	140	7 days
350	5 days	140	1 day
350	5 hours	140	4 days
350	1 hour	140	7 days
350	3 days	160	7 days
350	7 days	160	1 day
375	7 days	160	1 day
375	7 days	160	7 days
375	2 days	160	7 days
375	7 days	180	1 day
375	7 days	180	1 day
400	3 days	180	1 day
400	5 days	180	7 days
400	1 day	180	1 day
400	5 hours	190	3 hours
400	7 days	200	7 days
425	2 days	200	1 day
430	4 days	200	7 days
430	12 days	225	4 days
430	6 hours	265	5 hours
430	4 days	420	3 hours
600	10 minutes	450	1 hour
600	3 days		
700	1 day		
700	5 minutes		

Urea and sulfobromophthalein tests indicated some impairment of liver and kidney function during the experiment. However, immediately before the dogs were sacrificed, their liver and kidney function was normal. Urea determinations for the monkeys before sacrifice were slightly high but within the normal range. Pathological examinations of the animals revealed no significant gross or microscopic findings, only congestion.

Two dogs were given weekly intravenous ATA doses of 25 mg/kg. One was sacrificed after 8 months; the other died 2 days after the twentieth injection. The latter dog had lost weight during the previous 2 months and exhibited a high white count and high blood urea value. Pathological examination showed evidence of arteriosclerosis and severe congestion. The death was not due to the ATA treatment. No significant pathological findings other than congestion were noted in the animal that was sacrificed after 8 months of treatment.

Two monkeys were given 50 mg/kg of ATA weekly. After the ninth week both animals began to lose weight and abscesses were observed on their legs near the site of injection. Penicillin and streptomycin were administered to combat the infection. One of the monkeys died 2 days following the fourteenth ATA injection. Cultures from the leg abscess indicated a staphylococcus infection, but the heartblood was negative. The other monkey died 4 days after receiving the sixteenth injection. The primary microscopic finding indicated the same generalized congestion found in the other animals. However, the first animal that died after the fourteenth injection also showed moderate fatty metamorphosis of the liver cells.

D. Distribution and Elimination of ATA

The distribution and elimination of ATA in monkeys were investigated using ATA labeled at the central carbon atom. Four monkeys were given 100 mg/kg of ATA containing 0.1 millicurie of carbon-14 and placed in metabolism cages. Two of the animals were sacrificed 7 hr after injection and the other two 48 hr after injection. The animals were then autopsied and their tissues weighed. Tissue samples (1 to 3 g) were digested with formamide[3] until a clear solution was obtained and the solution was diluted with formamide to a final volume of 100 ml. A 1-ml aliquot of each digested tissue was added to 19 ml of scintillating solution. Color correction standards were made by adding labeled ATA to aliquots of unlabeled tissue digests. The diluted tissue solutions were counted in a Tri-Carb liquid scintillation counter.

Table 2 summarizes the results of this experiment. Although the entire injected dose was not recovered, it can readily be seen that the compound was rapidly cleared from the plasma.

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[3]Pearce, E. M., et al., Anal. Chem., Vol 28, p 1762, 1956.

Table 2

DISTRIBUTION OF ATA IN MONKEYS

Tissue	ATA Recovered, % After 7 Hr	After 48 Hr
Spleen	0.09	0.16
Heart	0.34	0.18
Kidneys	0.85	0.29
Stomach, small intestine	3.64	0.25
Liver	4.59	1.12
Lung	11.27	5.16
Plasma	30.82[a]	0.46[a]
Feces	0.06	1.18
Urine	-	0.66

[a]Calculations based on 6% total body weight.

E. Maximum Single Safe Dose of ATA

The estimated maximum safe dose was assumed to be 10% of the extrapolated zero mortality dose. Extrapolation of the regression curve from the dog data yielded a value of 59.5 mg/kg as the dose at which no deaths should occur. Therefore, the maximum safe dose would be 6 mg/kg. However, at this time, two dogs had been receiving 10 mg/kg per week for 12 weeks with no apparent harmful effects, so that the safety factor appeared too large. Thirty percent of the extrapolated value, 18 mg/kg, was then selected as the dose to be tested. This dose was given to ten dogs, and no deaths occurred. In view of the subsequent chronic experiment in which one dog tolerated 25 mg/kg per week for 8 months and another for almost 5 months, the maximum safe dose appears to be in excess of 25 mg/kg.

F. Acute Beryllium Sulfate Toxicity

The effects of the intravenous injection of $BeSO_4$ were studied in dogs and monkeys primarily to establish an approximate rather than an accurate LD_{50}. Six sets of two monkeys each were administered $BeSO_4$ at dose levels calculated to provide 0.2, 0.4, 0.5, 0.6, 0.7, and 0.8 mg/kg of Be. Six dogs which had been treated with 18 mg/kg of ATA 2 weeks previously were used to study Be toxicity since there were no apparent harmful effects. Two dogs were injected at each level of 0.4, 0.6, and 0.8 mg/kg of Be and two additional groups of two untreated dogs received 0.5 and 0.6 mg/kg. After the injections the animals were returned to their cages for 7 days of observation.

In contrast with the depression observed following ATA injections, the Be-treated animals were initially alert and active. Death generally occurred within 3 days following the injections. The results of the tests are given in table 3. These results indicate that Be doses above 0.6 mg/kg are fatal to both species. Subsequent experiments were based on these values.

Table 3

MORTALITY OF MONKEYS AND DOGS INJECTED INTRAVENOUSLY WITH BERYLLIUM SULFATE

Monkeys		Dogs	
Beryllium, mg/kg	Mortality*	Beryllium, mg/kg	Mortality*
0.2	0/2		
0.4	0/2	0.4	0/2
0.5	2/2	0.5	1/2
0.6	2/2	0.6	3/4
0.8	2/2	0.8	2/2

*Deaths/total.

Pathological examination of the dogs and monkeys that died as a result of the Be treatment also showed congestion of the internal organs. However, the changes in the liver were most remarkable. Severe fatty metamorphosis and hepatitis of the liver cells were observed. In addition, areas of necrosis surrounded by an inflammatory exudate were revealed in many of the liver slides. The finding of subcapsular hemorrhages in the kidney as well as diffuse lung hemorrhages was probably secondary to liver failure.

The acute toxicity of $BeSO_4$ following intratracheal injection was also studied. This method was used to simulate the inhalation of soluble Be compounds. In contrast to the use of aerosols, there is no loss of the material in the upper respiratory tract during intratracheal injection and the quantity deposited in the lungs can be precisely controlled and determined.

The animals were anaesthetized and the required dose was given by a needle inserted into the trachea below the larynx. The injections were timed to coincide with inspirations. The concentration of the injected solution was adjusted so that the total volume was less than 2 ml for monkeys and 5 ml for dogs. Monkeys and dogs in groups of two were given 1, 2, 4, 6, and 8 mg/kg of Be in this manner, and mortality was observed for a period of 7 days. As with the intravenous injections, the majority of deaths occurred within 3 days;

however, the responses of the two species were quite different as shown in table 4. Approximate LD_{50} values of 1.5 mg/kg of Be for monkeys and 2.0 mg/kg of Be for dogs were obtained by the Reed and Muench method.[4] Subsequent experiments were then based on intratracheal doses of 2 mg/kg for monkeys and 3 mg/kg for dogs.

Table 4

MORTALITY OF MONKEYS AND DOGS INJECTED INTRATRACHEALLY WITH BERYLLIUM SULFATE

Monkeys		Dogs	
Beryllium, mg/kg	Mortality*	Beryllium, mg/kg	Mortality*
1.0	0/2	1.0	1/2
2.0	2/2	2.0	2/2
4.0	2/2	4.0	3/4
6.0	2/2	6.0	1/2
8.0	2/2	8.0	3/4
		10.0	2/2
		12.0	2/2

*Deaths/total.

Pathological examination of the animals that received the soluble $BeSO_4$ via the trachea revealed the same general congestion and fatty degeneration of the liver as obtained upon intravenous administration. Since the material was deposited in the respiratory tract, more extensive lung damage was obtained. The sections revealed areas of diffuse hemorrhage in the alveolar lumina and septa, and in many instances these areas contained foci of necrosis. Thickening of the septa with scattered lymphocytic infiltration was also noted.

G. Beryllium Oxide Toxicity

Initial experiments to determine the acute toxic effects of BeO were made using aqueous suspensions of the insoluble material. One group of 5 mice and another of 10 mice were given 1 mg/kg by injection into the tail vein. There were no deaths in either of these groups. Similarly, a monkey survived an intravenous dose of 2 mg/kg of the suspension. Three other

[4] Reed, L. J. and H. Muench, Am. J. Hyg., Vol 21, p 493, 1938.

groups of 10 mice were injected intraperitoneally. For these injections the BeO was suspended in 0. 15% carboxymethylcellulose in water with the aid of a Vertis high-speed mixer. The three groups were given 1-ml injections containing 1, 10, and 100 mg/kg of Be, respectively. During the 7-day observation period no deaths occurred in the 1- and 10-mg groups, but 5 occurred in the 100-mg group. Microscopic histopathological examinations were not made of these animals. However, gross observations of animals in the 100-mg group that died or survived and were sacrificed at the end of the 7 day holding period revealed pale and quite congested livers. The other organs of these mice as well as all the organs of the ones that received the smaller doses appeared normal. The BeO was visible in the peritoneal cavity and appeared for the most part to have been localized in the area of the injection site.

Both dogs and monkeys were utilized for experiments in which BeO was given by intratracheal injection. For this purpose the animals were anaesthetized, and the BeO suspension containing 36 mg/ml of Be was injected into the trachea. Two monkeys were given 20 mg/kg of Be and one received 30 mg/kg. Two groups of two dogs each were also given 20 and 30 mg/kg of Be, respectively. None of the animals injected in this manner died.

The areas where the BeO was deposited in the lung were visible when the animals were sacrificed. Microscopic examination of tissues of a monkey sacrificed 26 days after the 20-mg dose revealed marked edema and fatty metamorphosis of the liver and pneumonitis of the lungs. However, one sacrificed 13 days after the 30-mg dose showed no microscopic changes in the liver. The lungs in this case were congested and contained areas in which the alveoli were filled with leucocytic exudate. Chronic inflammatory changes manifested by fibroblastic activity, lymphocytic infiltration, and foreign body giant cells were also observed.

These results indicate that high doses of BeO used in these studies did not produce acute toxicity when administered intravenously or intratracheally. However, the lung and liver pathology shows the possibility that the animals might eventually have died as a result of the administration of this material.

H. Treatment of Beryllium Poisoning with ATA

Several methods were used to evaluate the ability of ATA to prevent Be poisoning. In the experiments, dogs and monkeys were injected intravenously with combinations of ATA and Be doses ranging from 0 to 1.0 mg/kg of Be (as $BeSO_4$) and 0 to 200 mg/kg of ATA. In this series of experiments the ATA was administered 1 hr after the $BeSO_4$ injections and the mortality was observed for 7 days.

The results of these experiments are summarized in tables 5 and 6 for monkeys and dogs, respectively. The data in table 5 show a decrease in mortality for the monkeys that received 50 mg/kg of ATA at every toxic Be dose. The LD_{50} values were estimated by the Reed and Muench method for Be at 0 mg/kg ATA and at 50 mg/kg ATA and were found to be 0.63 and 0.87 mg/kg, respectively. No confidence limits can be placed on these values. Other statistical methods were utilized to ascertain the significance of the results in the individual treatment groups. Analysis of variance was performed on all ATA treatment groups at 0.8 mg/kg Be and also on the 0 and 50 mg/kg groups at this Be dose. The chi square test was employed to determine the significance between the 0 and the 50 mg/kg ATA groups at 0.8 mg/kg Be and between the 50 mg/kg ATA and the sum of all other treatment groups at this Be dose. In each instance the analysis indicated that the differences were significant at greater than the 99% probability level. Statistical analysis of the data in table 6 showed that ATA did not provide a significant degree of protection in dogs. The lack of protection may possibly be due to a smaller tolerance for ATA, as evidenced by an acute LD_{50} which is half that of the monkey.

Table 5

MORTALITY OF MONKEYS INJECTED WITH ATA 1 HR AFTER INJECTION WITH BERYLLIUM SULFATE

Beryllium mg/kg	ATA, mg/kg						
	0	25	50	75	100	150	200
	Mortality*						
0.0	0/3		0/3		1/3	1/3	0/2
0.4	0/5		0/3		0/3	0/3	
0.6	2/5		0/3		2/3	1/3	
0.8	5/5	3/3	1/5	3/3	3/3	1/3	
1.0	2/2		5/7	2/2	2/2		13/15

*Deaths/total.

Table 6

MORTALITY OF DOGS INJECTED WITH ATA 1 HR AFTER INJECTION WITH BERYLLIUM SULFATE

Beryllium, mg/kg	ATA, mg/kg						
	0	25	50	75	100	120	200
	Mortality*						
0.0	0/2	0/2	0/2		0/2		
0.4	1/4	0/2	1/2		1/2		
0.6	4/6	2/2	5/6		2/2		
0.8	4/4	2/2	6/8	2/2	2/2		
1.0			4/4			2/2	2/2

*Deaths/total.

Additional experiments were done in which ATA was administered at time intervals other than 1 hr after Be injections. In this series the Be dose was kept constant at 0.8 mg/kg. The results, summarized in table 7, show that these time intervals are not as effective as ATA given 1 hr after the Be.

Limited experiments were also performed to determine the ability of ATA to protect after intratracheal injection of $BeSO_4$. Two methods of ATA administration were used after Be doses of 2 mg/kg for monkeys and 3 mg/kg for dogs. In one series the required volume of 10% ATA to produce a 50-mg/kg dose was given to the animals 1 hr after the Be injections. One out of three monkeys given such treatment survived, and the only dog treated in this manner died. In the other series the animals received 25 mg/kg of ATA in a saline infusion 1 hr after the Be injection and another 25-mg/kg infusion 24 hr later. In this case one out of two monkeys and one out of three dogs survived.

I. Toxicity of ATA-Be Complex

The approach to treatment of Be poisoning used in this study is based on the premise that ATA chelation will reduce the effective concentration of Be ions to the extent that the toxic effects are minimized. Experiments were also conducted to determine whether the ATA-Be complex per se might be toxic.

Table 7

MORTALITY OF MONKEYS AND DOGS INJECTED WITH ATA AT VARIOUS INTERVALS AFTER INJECTION WITH BERYLLIUM SULFATE

Animal	Beryllium, mg/kg	ATA, mg/kg	Treatment Interval	Mortality*
Monkey	0.8	45**	3 successive days	3/3
Monkey	0.8	50	8 hr after Be	3/3
Monkey	0.8	40	Simultaneous***	1/3
Dog	0.8	25	3 successive days	1/2
Dog	0.8	50	Simultaneous***	2/2
Dog	0.8	50	8 hr after Be	2/2

*Deaths/total.

**Total ATA dose, given as 20, 15, and 10 mg/kg on successive days.

***Be and ATA given simultaneously in different limbs.

The complex was prepared by mixing solutions of $BeSO_4$ and ATA and adjusting the pH to 7.3. The insoluble lake produced was treated in the Vertis mixer or with 14-kc sonic energy to achieve a homogeneous mixture of small particles. Numerous combinations of Be and ATA were used for the intravenous injections. All four monkeys died after receiving quantities of the mixture containing 1 mg/kg of Be at a Be-to-ATA weight ratio of 1 to 100. Two dogs treated with the same concentration also died. Similarly, four monkeys and two dogs died when the Be dose was kept at 1 mg/kg and the quantity of ATA in the complex was reduced to 50 mg/kg. Two monkeys died when the dose contained 0.8 mg/kg of Be and 50 mg/kg of ATA, a weight ratio of 1 to 62.5. However, one out of two monkeys survived when the mixture contained 0.6 mg/kg of Be and 50 mg/kg of ATA, a weight ratio of 1 to 83.3. The last two complex mixtures corresponded to the Be and ATA doses at which protection was obtained in the experiment on ATA therapy.

Death upon injection of the complex usually occurred immediately after and in some cases before the injection was completed. Convulsivelike movements were also observed in these animals. These observations led to the hypothesis that the deaths were not due to pharmacological changes produced by the complex but rather to mechanical blockage of some of the smaller blood vessels. To test this hypothesis, one monkey was given simultaneously 0.4 mg/kg of Be in one leg and 25 mg/kg of ATA in the other leg. This combination should not produce death when the injections are separated by 1 hr (Table 3), and this monkey also survived. Another monkey was given the same

concentration of Be and ATA but in the form of the complex. This animal died within 20 min of the injection. Gross observations on necropsy revealed several blood vessels in the lateral ventricle that appeared to contain emboli. These results indicate that the complex probably cause death by mechanically blocking the blood vessels.

An attempt was made to determine whether there are any additional toxic properties in the complex. Mice were given the complex by intraperitoneal injection. Any toxic effects observed should be due to absorption from the peritoneal cavity without the complication of obstruction of the blood vessels found in intravenous administration. Ten mice were given the 1:50 complex equivalent to 1 mg/kg of Be, ten were given 1 mg/kg of Be intraperitoneally, and ten were given 1 mg/kg of Be intraperitoneally and 50 mg/kg of ATA 1 hr later via the tail vein. None of the mice that received both Be and ATA died, two that received Be alone died, and three that received the complex died. These results indicate that the complex may also be acting pharmacologically.

III. DISCUSSION

The results obtained during these studies indicate that ATA treatment affords some protection against Be poisoning in monkeys. However, there are many aspects of the interaction between ATA, Be, and tissue proteins that must be investigated before any attempt is made to use ATA treatment on a clinical basis. Schubert et al.[2] ascribed the therapeutic effectiveness to the formation of a stable nontoxic lake between ATA and Be in the tissues rather than to an increased excretion or redistribution of Be. This lake formation inactivates the Be ions in situ and is stated to contain two molecules of ATA for each Be ion. A molar ratio of 2 to 1 corresponds to a weight ratio of 105 to 1 using the ammonium salt of ATA. In the initial experiments, this value was doubled and the animals were given 200 mg/kg of ATA after a 1-mg/kg Be injection. Only two of the fifteen monkeys and neither of the two dogs treated in this manner survived. Thus, increasing the ATA levels above the stoichiometric ratio is not effective.

A block design was used for the remainder of the 1-hr treatments so that any beneficial results could be directly compared to the results from animals receiving Be alone or other ATA concentrations at an particular Be dose.

The initial results for both dogs and monkeys were unexpected in that the best protection at 0.8 mg/kg was obtained with 50 mg/kg of ATA, which is less than the stoichiometric ratio. This dose was also effective for two of the three monkeys first studied at 1.0 mg/kg of Be, but there were no other survivors in subsequent experiments. Similarly the 50-mg/kg ATA dose was the only one effective in initial experiments with dogs, but again there were no survivors in the later experiments. No additional experiments were made with the 150-mg/kg ATA dose in monkeys after a 0.8-mg/kg dose. One animal died after 3 days, which was the usual survival time for acute Be poisoning within the 7-day observation period. The other animals died after 10 and 13 days.

Although the intratracheal dose of Be given to monkeys was 2.5 times the intravenous dose, 2 out of 5 animals treated with 50 mg/kg of ATA survived.

Mukherji and Dey,[5] on the basis of spectrophotometric and conductivity measurements, concluded that a 1:1 chelate is formed in the $BeSO_4$-ATA system. They suggested that chelation occurs between a phenolic oxygen and the adjacent carboxylic oxygen. Schubert[2] suggests the same bonds between the Be ion and ATA but indicates that two ATA molecules are attached to each Be ion. Other workers[6,7] have shown that at physiological pH the Be ion exists as $BeOH^+$ rather than Be^{++}. This ionic species would be more likely to form a 1:1 chelate with ATA rather than one containing two molecules of ATA for each Be. Chelation in this manner would explain the protection at 50 mg/kg rather than 100 mg/kg.

The efficacy of ATA treatment appears to be related to the time interval between Be intoxication and ATA administration. Schubert[2] found no difference in the protective action of ATA given 5 min or 1 hr after Be poisoning in mice. ATA proved effective as late as 8 hr after Be injection but was ineffective after 16 hr.[8] Our results show that, to be effective, ATA treatment must be given within a reasonably short time interval after the Be injection. The treatment schedule which proved effective 1 hr after the injection was not effective 8 hr after Be injection.

These studies show that the use of ATA in the treatment of accidental exposure of humans to Be appears feasible if it can be demonstrated that the effective ATA dose produces no adverse effects. In the case of Be exposures suspected to be lethal, ATA treatment is advised because it offers the only possible means of preventing death.

IV. SUMMARY

The use of 50 mg/kg of ATA as a therapeutic dose was effective in protecting monkeys exposed to lethal quantities of Be. This effectiveness was confirmed by statistical analysis of the experimental data. The analysis of variance and the chi square test ascertained the significance of the results at probability levels higher than 99%. The effectiveness of ATA treatment appears to be related to the time interval between exposure to Be and the administration of ATA. Although detailed studies of the time schedule were not conducted, it appears that the time interval should be kept to approximately 1 hr.

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[5] Mukherji, A. K. and A. K. Dey, Z. physik. Chem., Vol 210, p 114, 1959.

[6] Scheel, L. D., Personal communication.

[7] Adamovich, L. P. and G. S. Shopenko, Ukrain, Khim. Zhur., Vol 25, p 155, 1959.

[8] White, M., A. Finkel, and J. Finkel, and J. Schubert, J. Pharmacol. Exptl. Therap., Vol 102, p 88, 1951.

The ATA treatment was not successful in treatment of dogs exposed to lethal doses of Be.

Although a significant degree of protection was obtained in monkeys, additional in vivo and in vitro studies should be conducted before recommendations can be made for the use of ATA in treatment of humans accidentally exposed to Be.

APPENDIX

REPRESENTATIVE SLIDES OF TISSUES FROM ANIMALS TREATED WITH BERYLLIUM AND ATA

The samples were fixed in 10% buffered formalin and stained after sectioning with hematoxylin and eosin. The microscopic description of the tissues is as follows.

MC21 - Monkey given weekly injections of 25 mg/kg of ATA for 8 months

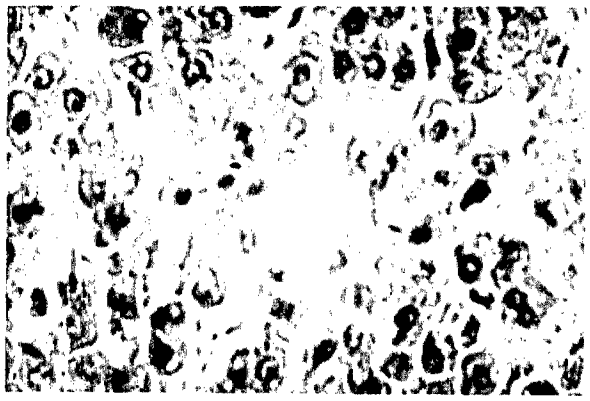

Figure 1. MC21 - LIVER

No significant change except for marked congestion.

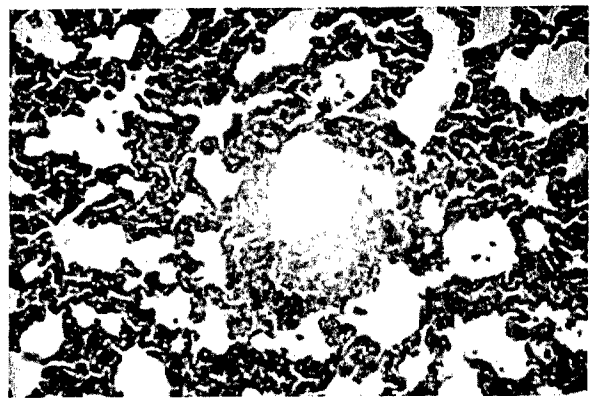

Figure 2. MC21 - LUNG

Marked passive congestion.

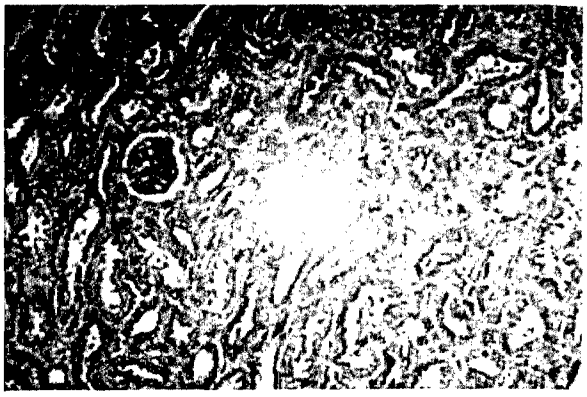

Figure 3. MC21 - KIDNEY

No significant change, scattered lymphocytes in interstitium.

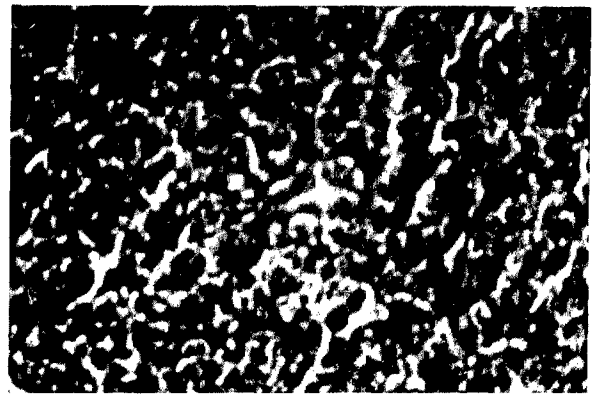

Figure 4. MC21 - SPLEEN

No significant change.

DB37 - Dog sacrificed 10 days after receiving 100 mg/kg of ATA

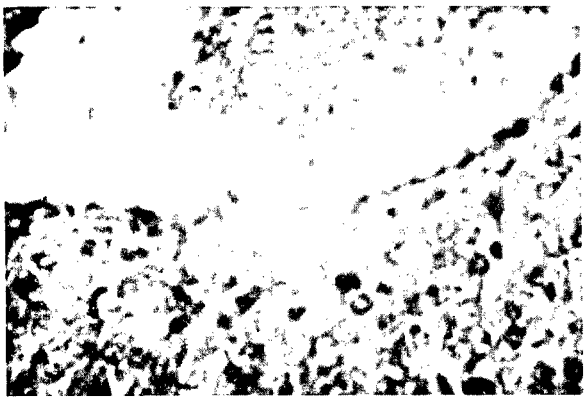

Figure 5. DB37 - LIVER

Marked dilation of the central vein intralobular sinuses.

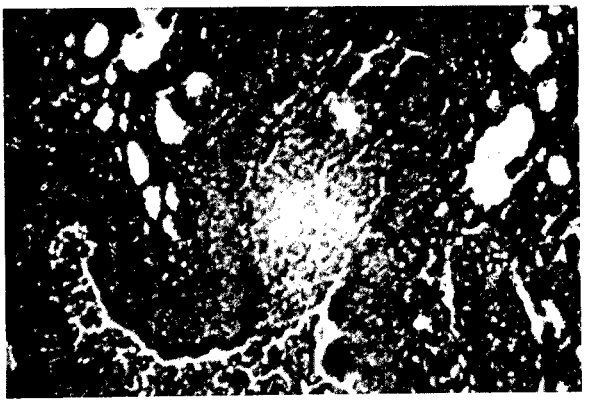

Figure 6. DB 37 - LUNG

The veins and the capillaries are distended with blood. The septa are thickened and contain an accumulation of round cells and scattered leucocytes. Small bronchi contain a leucocytic exudate.

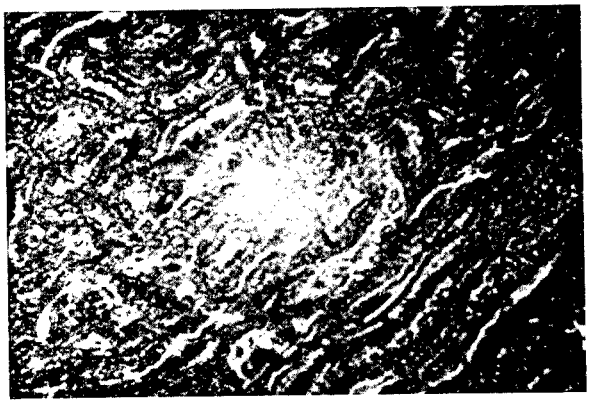

Figure 7. DB37 - KIDNEY

Marked congestion and degenerative changes of tubular epithelium.

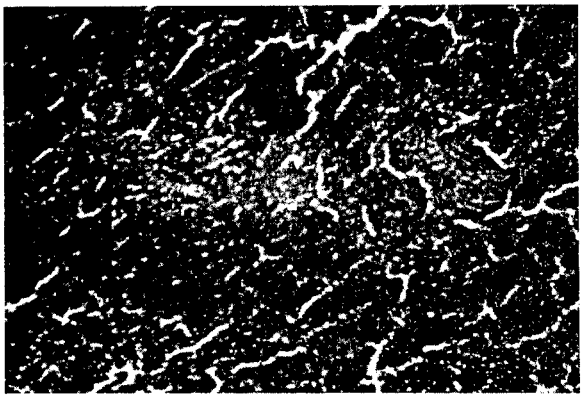

Figure 8. DB37 - SPLEEN

Dilation of the sinusoids.

MB3 - Monkey died 3 days after receiving 0.6 mg/kg of beryllium intravenously

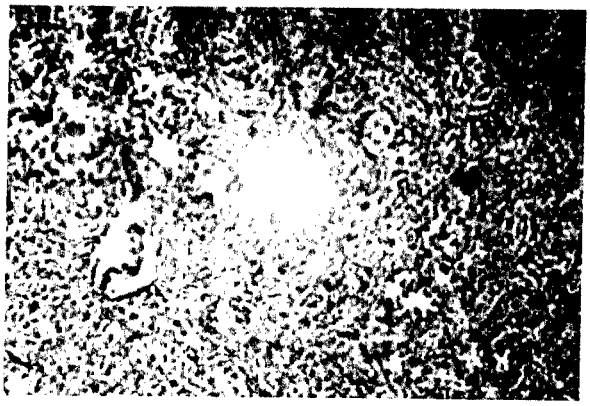

Figure 9. MB3 - LIVER

Marked fatty metamorphosis of the liver cells. Edema of the periportal spaces with moderately severe inflammatory exudate.

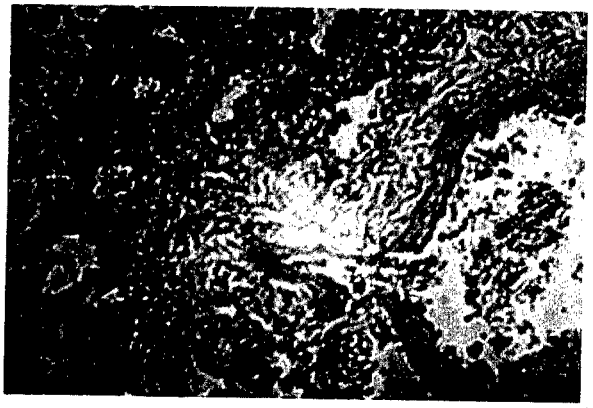

Figure 10. MB3 - LUNG

Congestion throughout and an intra-alveolar exudate in small groups of alveoli.

MB157 - Monkey died 3 days after receiving 2 mg/kg of beryllium intratracheally

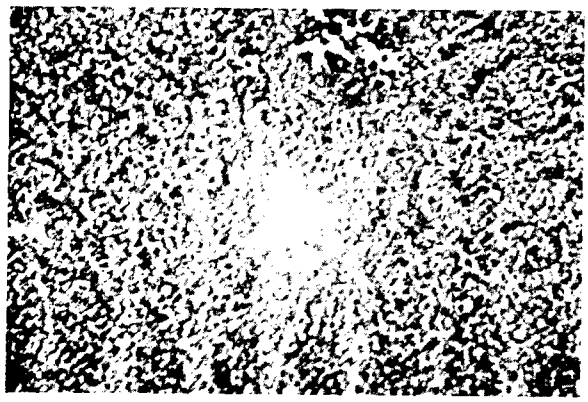

Figure 11. MB157 - LIVER

Congestion and fatty metamorphosis.

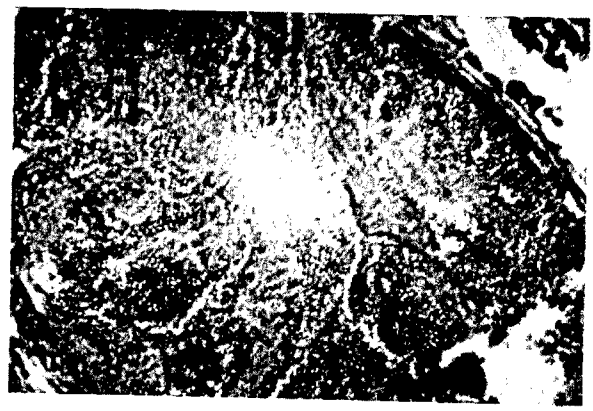

Figure 12. MB157 - LUNG

Marked congestion. Recent thrombus in a small branch of the pulmonary vessel, thickening of the septa with lymphocytic infiltration.

MB175 - Monkey sacrificed 26 days after receiving 20 mg/kg of beryllium as beryllium oxide intratracheally

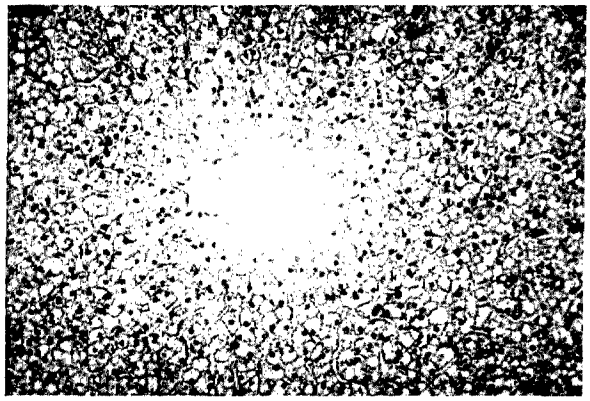

Figure 13. MB175 - LIVER

Congestion, fatty infiltration, and edema of periportal spaces.

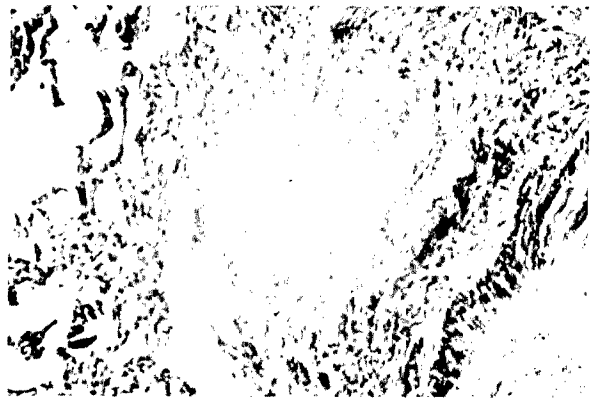

Figure 14. MB175 - LUNG

Thickening of the septa, early lymphocytic and leucocytic infiltration.

DB14 - Dog sacrificed 3 months after receiving 0.6 mg/kg of beryllium intravenously, followed in 1 hr by 60 mg/kg of ATA

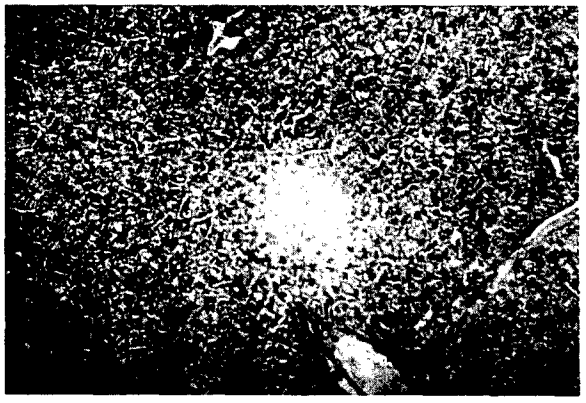

Figure 15. DB14 - LIVER

Congestion, some vacuolization of the cytoplasm and a few fat droplets.

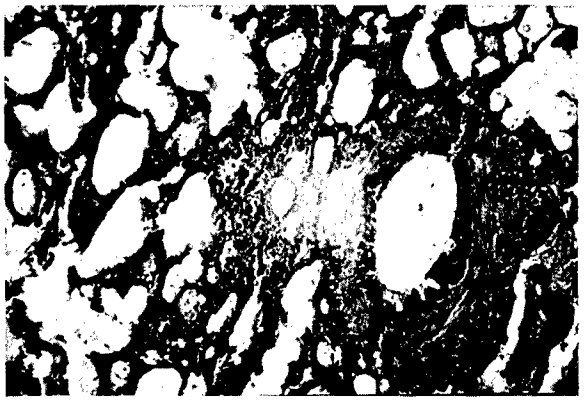

Figure 16. DB14 - LUNG

Marked congestion and thickening of the septa.

Figure 17. DB14 - KIDNEY

No significant change.

Figure 18. DB14 - SPLEEN

Marked fibrosis of the trabeculae and hemosiderin deposits.

DB200 - Monkey died 2 days after receiving 1 mg/kg of beryllium intravenously followed in 1 hr by 50 mg/kg of ATA

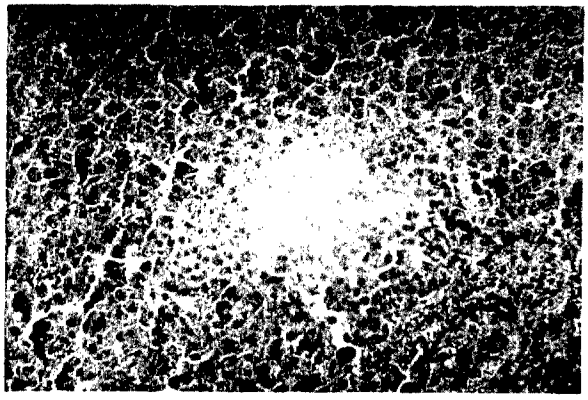

Figure 19. DB200 - LIVER

Severe congestion, slight fatty metamorphosis.

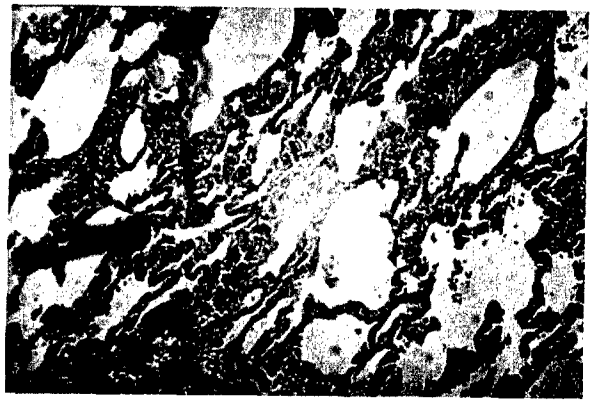

Figure 20. DB200 - LUNG

Marked congestion, serous exudate within alveolar lumina, foci of hemorrhages.

Figure 21. DB200 - KIDNEY

Marked congestion.

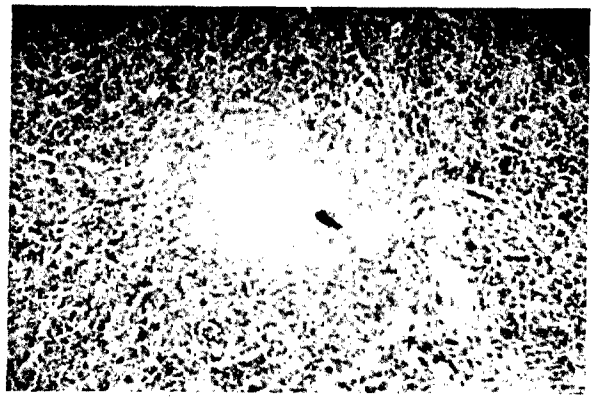

Figure 22. DB200 - SPLEEN

No significant change.

MB25 - Monkey died immediately following 1:50 complex

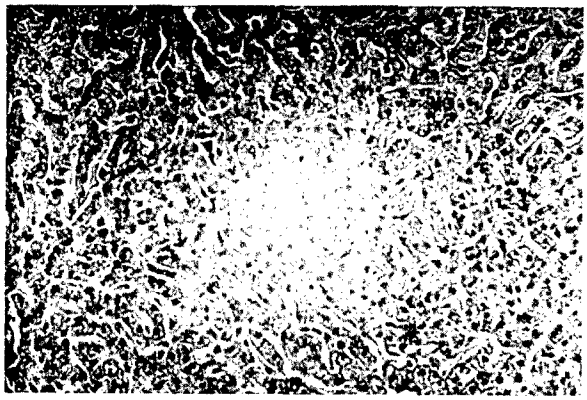

Figure 23. MB25 - LIVER

Congestion.

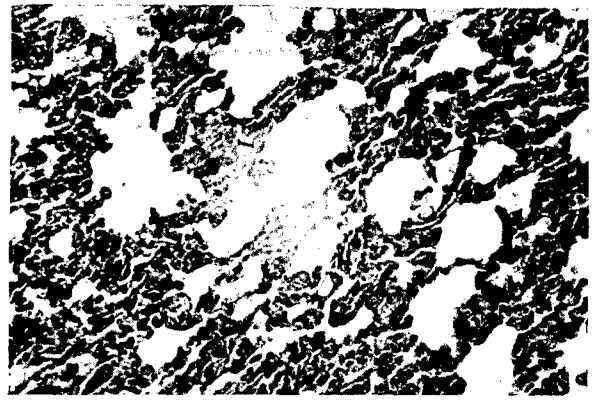

Figure 24. MB25 - LUNG

Marked congestion, thickening of the septa, and focal accumulations of lymphocytes.

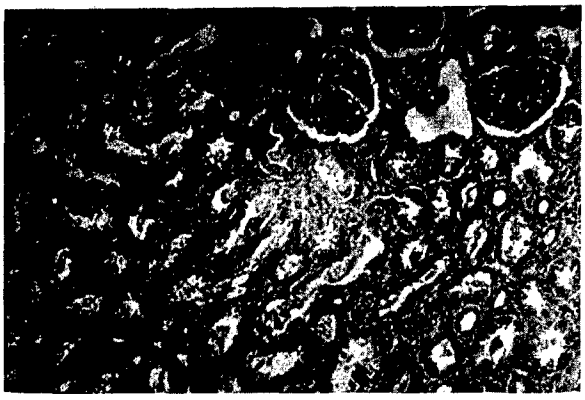

Figure 25. MB25 - KIDNEY

Congestion.

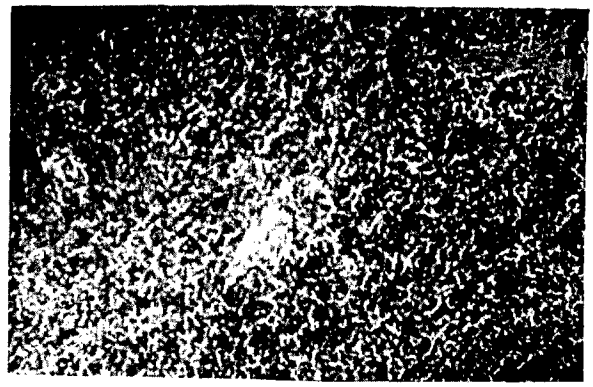

Figure 26. MB25 - SPLEEN

Thickening of the trabeculae and hemosiderin deposits in the pulp.

ASD TR 61-674

Armour Research Foundation, Illinois Institute of Technology, Technology Center, Chicago 16, Illinois

TOXICOLOGY OF AURIN TRICARBOXYLIC ACID AND ITS ANTIDOTAL EFFECTIVENESS AGAINST BERYLLIUM, by M. E. King. December 1961. 31p. incl. illus., tables. 8 refs. (Proj. 7165; Task 716501)

Unclassified report

Monkeys and dogs were used in a series of studies designed to assess the ability of aurin tricarboxylic acid (ATA) to provide protection against acute beryllium poisoning. The acute LD_{50} of ATA was found to be 344 mg/kg for monkeys and 164 mg/kg for dogs. Neither species exhibited significant (over)

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I. King, M. E.
II. Aeronautical Systems Division, Aerospace Medical Laboratory, Wright-Patterson Air Force Base, Ohio
III. Contract No. AF 33(616)-6947

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hematological changes when given weekly ATA doses of 25 mg/kg over an 8-month period. The lethal intravenous dose of beryllium sulfate was 0.6 mg/kg for both dogs and monkeys, but the value increased to between 1 and 3 mg/kg when given by intratracheal injection. Acute toxic effects were not observed by either intravenous or intratracheal doses of suspensions of beryllium oxide. Treatment with ATA appeared to have therapeutic value in monkeys exposed to beryllium, but no significant response was observed in dogs.

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